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(21) International Application Number: PCT/GB90/01443 (22) International Filing Date: 19 September 1990 (19.09.90) (30) Priority data: 8921123.9 19 September 1989 (19.09.89) GB (71)(72) Applicant and Inventor: MILLAR, Ann, Brigid [IE/GB]; Department of Medicine, University College and Middlesex School of Medicine, The Middlesex Hospital, London W1N 8AA (GB). (74) Agent: HALLYBONE, Huw, George; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).		(81) Designated States: AT (European patent), AU, BE (European patent), CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB, GB (European patent), IT (European patent), JP, KR, LU (European patent), NL (European patent), SE (European patent), US. Published With international search report.
(54) Title: TREATMENT OF ARDS		
(57) Abstract Patients experiencing adult respiratory distress syndrome (ARDS) have elevated levels of tumour necrosis factor- α (TNF) in their bronchoalveolar lavages. This is so even in the absence of overt sepsis. Antibody to TNF (Anti-TNF) may therefore be an effective treatment for ARDS, especially when administered directly to the lung surface, for example as an aerosol.		

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Treatment of ARDS

Technical Field

The present invention relates to the treatment of adult respiratory distress syndrome (ARDS), a medicament for the treatment of ARDS, and to the manufacture of such a medicament.

Background Art

ARDS is a descriptive term which is applied to a variety of acute, diffuse, lung lesions which, while having differing aetiologies, have similar pathology and clinical characteristics (16). Increase in respiratory frequency, decrease in tidal volume and in lung compliance and deterioration in gas exchange are all symptomatic of the syndrome and result in arterial hypoxaemia.

In many cases ARDS is associated with septicaemia (septic-ARDS) and mortality of sepsis in patients with ARDS is greater than 80%(5). However, ARDS is not, invariably, associated with the symptoms of sepsis.

Where sepsis is present endotoxin, the lipopolysaccharide (LPS) component of the cell wall of Gram-negative bacteria, acts as a potent macrophage activator and induces the synthesis and secretion of biologically active molecules including cytokines. One of these, tumour necrosis factor- α (TNF) is in turn responsible for the release of vasoactive peptides, platelet activating factor, interleukin-8, prostaglandins and other cytokines which mediate inflammatory and acute phase responses (11). TNF has been postulated to have an important pathophysiological role in a number of clinical situations including septic shock and malignant disease (1-4).

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Elevated serum levels of TNF have been detected in patients with septicaemia and have a predictive value in relation to outcome (4). More recently TNF has been postulated as a mediator in the development of ARDS in septic patients (5). The main evidence for this suggestion comes from animal models (5,6). The prior administration of anti-TNF monoclonal antibodies has been shown to protect animals from the lethal effects of induced sepsis (12,13) and the treatment of septic ARDS with antibody to TNF (anti-TNF) has been suggested (5).

The role of TNF in ARDS in those patients who show no symptoms of sepsis has remained unclear. Furthermore, existing studies have failed to establish the intrapulmonary, as opposed to systemic, role of TNF either in the presence or absence of sepsis. One study showed that levels of plasma TNF showed no correlation with the development of ARDS in a prospective study of patients with sepsis (15).

The present applicant has determined the levels of TNF present in the bronchopulmonary secretions of the lung in patients with ARDS but showing no overt indication of sepsis at the time of sampling the secretions. Surprisingly, high levels of intrapulmonary TNF were found in these patients.

Furthermore, blood samples from patients who had undergone cardiopulmonary bypass operations, and who are particularly susceptible to an ARDS like condition, were examined and shown to produce increased TNF relative to control samples. Animal experiments confirmed the causative role of TNF in inducing lung damage.

Disclosure of Invention

According to a first aspect of the present invention there is provided the use of an antibody to TNF- α (anti-TNF) in the manufacture of a medicament for the treatment of or prevention of non-septic ARDS. By non-septic ARDS is meant ARDS which is not associated with overt sepsis at the time of diagnosis and/or treatment. In particular, the medicament is for treatment of ARDS which is not associated with overt sepsis either at the time of diagnosis or of treatment. The characteristics of overt sepsis are well known to the clinician. For example, a patient with overt sepsis may have a temperature below 36°C or above 38.5°C together with an abnormal white blood cell count of say below 3×10^9 cells/L or above 9×10^9 /L. Furthermore, there is generally a suspected source of infection.

According to a second aspect of the invention there is also provided a method of treatment of a human or animal subject suffering from or at risk from non-septic ARDS the method comprising administering an effective amount of an anti-TNF antibody.

Examples of conditions and situations which may lead to non-septic adult respiratory distress syndrome, or in conjunction with which non-septic ARDS may occur, and where a medicament manufactured according to the method of the present invention may be of utility include: diffuse pulmonary infections, such as viral, bacterial or fungal infections or protozoal infections such as pneumocystis; aspiration of liquid; inhalation of toxins and irritants; drug overdose; cardiopulmonary bypass; immunological responses to host or other antigens, for example as occurs in Goodpasture's syndrome or systemic

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lupus erythematosis; and nonthoracic trauma together with hypotension, for example as occurs in "shock lung".

The anti-TNF antibody for use according to the invention may, in general, belong to any immunoglobulin class or subclass. Thus, for example, the anti-TNF antibody may be an immunoglobulin G or immunoglobulin M antibody.

The anti-TNF may be of animal, for example mammalian, origin and may be, for example, of murine, rat, hamster or human origin. The antibody may be a whole immunoglobulin, or a fragment thereof, for example a F(ab')₂, Fab or Fv fragment.

The anti-TNF antibody may be polyspecific but is preferably monospecific for human TNF- α . The antibody may be a polyclonal antiserum or a monoclonal antibody. Particularly useful antibodies for use according to the invention include recombinant anti-TNF antibodies, i.e. anti-TNF antibodies which have been produced using recombinant DNA techniques. Especially useful antibodies of this type are antibodies having an antigen binding site at least part of which is derived from an immunoglobulin from a non-human species, the remainder of the molecule being derived from a human immunoglobulin.

The anti-TNF antibody may be prepared using well-known immunological techniques employing TNF- α as antigen. Thus, for example, any suitable host may be injected with TNF- α and the serum collected to yield the desired polyclonal anti-TNF antibody after appropriate purification and/or concentration, (for example by affinity chromatography using immobilised TNF- α as the affinity medium).

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Alternatively, splenocytes or lymphocytes may be recovered from the TNF- α -injected host and immortalised using for example the method of Kohler *et al.*, Eur. J. Immunol. 6, 511, (1976), the resulting cells being segregated to obtain a single genetic line producing monoclonal anti-TNF α antibodies in accordance with conventional practice. Antibody fragments may be produced using conventional techniques, for example by enzymatic digestion e.g. with pepsin [Parham, J. Immunol., 131, 2895, (1983)] or papain [Lamoyi and Nisonoff, J. Immunol. Meth., 56, 235, (1983)]. Where it is desired to produce recombinant anti-TNF α antibodies these may be produced using for example the methods described in European Patent Specifications Nos. 171496, 173494, 194276 and 239400.

In order to treat non-septic ARDS the anti-TNF antibody may, in general, be administered in any appropriate form. For example anti-TNF may be administered intravenously together with a pharmaceutically acceptable carrier, excipient or diluent. Such a composition may be in a form suitable for bolus injection or continuous infusion. Compositions for injection may take such forms as suspensions, solutions or emulsions of anti-TNF in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents.

In view of the inventor's observation that there are high, localised concentrations of pulmonary TNF the antibody may also, suitably, be administered locally to the lungs by instillation or as a dispersion or aerosol, for example in nebulised form or as liposomes. Thus, the invention also provides, according to a further aspect, a pharmaceutical composition for the treatment of ARDS the composition comprising anti-TNF and a pharmaceutically acceptable carrier, excipient or diluent the composition being

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arranged for administration as an aerosol. Use of anti-TNF in the manufacture of such a pharmaceutical composition is provided according to a still further aspect of the invention. Such a composition is suitable for the therapy of patients with either septic or non-septic ARDS.

Additionally, according to a still further aspect of the invention there is provided an aerosol comprising anti-TNF and a pharmaceutically acceptable excipient, diluent or carrier. The aerosol may be produced by means of a nasal spray containing a pharmaceutical composition comprising anti-TNF and a pharmaceutically acceptable excipient, diluent and carrier; and further having means for aerosolising the pharmaceutical composition.

Pharmaceutical compositions for the treatment of ARDS may contain, in addition to anti-TNF antibody further active ingredients.

The compositions may be used to treat an existing condition or, alternatively, may be used prophylactically to prevent development of ARDS in patients who may be particularly susceptible to the syndrome e.g. those who have had cardiopulmonary bypass surgery.

The dose at which the anti-TNF antibody will be administered will depend on the nature and severity of the condition. Local concentrations of TNF may be measured and a suitable dose of anti-TNF then selected by the physician. In general, the total dose of anti-TNF will be not less than 0.1 mg/kg per day and will not exceed 80 mg/kg per day. Where the antibody is administered by infusion a dose in the range 0.1 - 20 mg/kg may be administered one to four times a day. For example a single infusion of about 10 mg/kg is suitable .

Examples

The following examples serve to illustrate that an anti-TNF antibody would be of utility in the treatment of non-septic ARDS. In Example 1 patients suffering from ARDS but who were not overtly septic at the time of examination were shown to have elevated levels of TNF in bronchoalveolar lavages. In Example 2 TNF was employed in an animal model and shown to result in pulmonary damage. Finally, in Example 3, TNF production by the blood cells of patients who had undergone cardiopulmonary bypass was investigated. Such patients are particularly susceptible to a non-septic ARDS-like syndrome.

Example 1

To ascertain whether tumour necrosis factor (TNF) was present within the bronchopulmonary secretions of patients with adult respiratory distress syndrome (ARDS), five patients with this condition were studied. Each patient underwent fiberoptic bronchoscopy and bronchopulmonary aspiration. Control samples were obtained in an identical manner from twenty-four patients undergoing bronchoscopy of whom eight had tuberculosis, six had sarcoidosis and ten had no abnormal findings. The aspirated fluid was assayed for the presence of TNF using an enzyme linked immunoabsorbance technique (ELISA). TNF levels of greater than 500 u/ml (12.5 ng/ml) were detected from the five patients with ARDS, whereas in the control samples no TNF was detected. These data suggest that intrapulmonary TNF production may be involved in the development of ARDS, and that anti-TNF would be a suitable treatment for the condition.

Methods:

The subjects were 5 adult patients requiring intensive care and mechanical ventilation for respiratory failure. All the patients had ARDS as defined by Ashbaugh (7). They all had an accepted risk factor, an arterial oxygen tension (kPa) to inspired oxygen tension ratio of greater than 20, bilateral widespread pulmonary infiltrates on chest radiographs and pulmonary calpillary wedge pressures of less than 15mmHg. Their mean age was 56 ± 9 yr. The conditions leading to admission of the patients to ITU were as follows, severe burns and smoke inhalation, ureteric rupture, faecal peritonitis, pancreatitis, and massive blood transfusion. At the time of the study none of the patients had been overtly septic for at least 48hr i.e. they were afebrile, had white blood cell count between 3 and $9 \times 10^9/L$ and their routine swabs were sterile. The control specimens were from 24 patients undergoing bronchoscopy as part of their clinical assessment. Six of them had tuberculosis, 5 had sarcoidosis and 10 were being investigated for haemotysis but had normal bronchochoscopies and negative microbiological and cytological samples. Their mean age was 65 ± 13 yr.

In each patient fibreoptic bronchoscopy was performed and in the ARDS group this was via the endotracheal tube. Local anaesthesia with lignocaine (2%) was used in all cases. Any bronchopulmonary secretions below the trachea (and endotracheal tube) were aspirated and then 20ml of physiological saline at 37°C was instilled and aspirated. The total resultant aspirate was transported to the laboratory on ice and rapidly frozen at -20°C. All the control patients subsequently underwent formal bronchoalveolar lavage (BAL) (8) and a sample of this fluid was treated and stored in an identical manner. All the samples were assayed within a week of collection.

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The bronchopulmonary secretions and BAL fluid were tested for the presence of TNF using an enzyme linked immunoabsorbent assay (ELISA) with a double sandwich layer technique. This assay utilised 2 mouse anti-human monoclonal antibodies as previously described (9,10). Titres are expressed in units of activity in direct proportion to those of an interim TNF standard, assigned 40,000 units per gm of recombinant human TNF. The detection limit of the assay was 10u/ml.

Results:

The mean level of TNF detected in the patients with ARDS was 523 ± 186 u/ml (13.1 ± 4.6 ng/ml) (Table 1). By contrast, no TNF was detected in either the bronchopulmonary secretions nor the BAL fluid of the control subjects.

Table 1

Patient	Diagnosis	TNFu/ml
1	burns	610
2	pancreatitis	465
3	massive blood transfusion	375
4	sepsis	530
5	ureteric rupture	846

It is noteworthy that previously published data on plasma levels of TNF in septic patients report TNF levels of less than 1ng/ml and death occurred in all patients in whom TNF levels greater than 0.1 ng/ml were observed. Four of the patients in the present study survived despite intrapulmonary levels of more than 13ng/ml which makes a systemic source unlikely.

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The present findings suggest that local pulmonary TNF production is important in the pathogenesis of ARDS. The therapeutic use of anti-TNF antibodies might have a role in patients at risk of and with established ARDS, where either intravenous or aerosolised delivery might be appropriate.

Example 2

The intravenous injection of human recombinant TNF (50-150 μ g/kg) into rabbits caused noticeable respiratory distress with a significant fall in blood PO₂. Lungs removed from animals 4 hours after TNF administration were fixed, processed and stained for histological examination.

The small airway tissue showed marked signs of alveolar inflammation with microcirculatory congestion, interstitial thickening and infiltration of inflammatory leucocytes. In a group of animals receiving the anti-TNF Mab, CB6 (10mg/kg i.v.) 30 min before TNF challenge, there were no respiratory changes and the histology of the lungs did not differ from the lungs of a control group of animals injected with saline instead of TNF.

These observations support the hypothesis that TNF has a causative role in the pathology of non-septic ARDS, and that an anti-TNF antibody would be effective in amelioration of the syndrome.

Example 3

Eight patients undergoing coronary artery bypass grafting were studied, with a mean age of 59 ± 6 years. All the patients had no previous history of lung disease, normal chest X-rays and had stopped smoking for a minimum of six months prior to surgery. Each patient had blood taken pre and postoperatively and fiberoptic bronchoscopy (FOB) and bronchoalveolar lavage (BAL) post-surgery, whilst still ventilated. Four control patients undergoing vascular surgery were studied as controls and had blood taken pre- and postoperatively. Monocytes and macrophages were separated from blood and BAL fluid respectively by adherence to plastic. These cells were cultured alone and with lipopolysaccharide. The cell culture supernatants were assayed for TNF using a double sandwich ELISA method.

The results (mean \pm standard deviation, iu/ml) are shown below at Table 2. They suggest that in these patients cardiopulmonary bypass increases the spontaneous and stimulated production of TNF from peripheral blood monocytes but has no direct effect on alveolar macrophages.

The cardiopulmonary bypass patients in this study did not develop ARDS, despite the fact that elevated production of TNF in the peripheral blood circulation was observed. The results suggest that in those patients who do develop ARDS some further stimulus is present which triggers TNF production by lung macrophages.

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Table 2

		Cardiopulmonary Bypass		Controls	
		pre- operative	post- operative	pre- operative	post operative
Monocytes	Spontaneous	16 \pm 15	58 \pm 42	18 \pm 12	20 \pm 10
	Stimulated	62 \pm 43	217 \pm 47	57 \pm 34	61 \pm 31
Macrophages	Spontaneous	-	21 \pm 11	-	-
	Stimulated	-	51 \pm 23	-	-

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- 15) Marks JD, Luce JM, Montgomery AB et al. The presence of tumor necrosis factor in patients with septic shock who develop the adult respiratory distress syndrome. Am Rev resp Dis 1988; 138: 229 (abstract)
- 16) Ingram Jr, RH. Adult respiratory distress syndrome; Harrison's Principles of Internal Medicine 10th Edn - 1983; 1592-1595

CLAIMS

1. Use of an antibody to TNF- α (anti-TNF) in the manufacture of a medicament for the treatment or prevention of non-septic ARDS.
2. A use according to Claim 1 wherein non-septic ARDS occurs in association with, or as a consequence of, one or more of the following: diffuse pulmonary infection; aspiration of liquid; inhalation of a toxin or irritant; drug overdose; cardiopulmonary bypass, immunological response to host antigen; non-thoracic trauma associated with hypotension.
3. A pharmaceutical composition for the treatment of septic or non-septic ARDS, the composition comprising anti-TNF and a pharmaceutically acceptable excipient, diluent or carrier, the composition being arranged for administration as an aerosol.
4. Use of anti-TNF in the manufacture of a medicament for the treatment of septic or non-septic ARDS, the medicament being for administration directly to the lung.
5. A use according to Claim 4 wherein the medicament is for administration as an aerosol.
6. An aerosol comprising anti-TNF and a pharmaceutically acceptable excipient diluent or carrier.
7. A nasal spray apparatus comprising: a container and, inside the container, a pharmaceutical composition comprising anti-TNF and a pharmaceutically acceptable excipient, diluent, or carrier; the container being provided with means for aerosolising the composition.

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8. A method for the treatment or prevention of non-septic ARDS, the method comprising administering, to a human or animal subject suffering from, or at risk from, non-septic ARDS, an effective amount of an anti-TNF antibody.
9. A method for the treatment or prevention of septic or non-septic ARDS, the method comprising administering to a human or animal subject suffering from, or at risk from ARDS, an effective amount of an anti-TNF antibody, administration of the TNF being direct to the lung of the subject.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01443

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶ According to International Patent Classification (IPC) or to both National Classification and IPC IPC ⁵ : A 61 K 39/395, A 61 K 9/12																	
II. FIELDS SEARCHED <div style="text-align: center; margin-top: 10px;">Minimum Documentation Searched ⁷</div> <table style="width: 100%; border: none;"> <tr> <td style="width: 25%; border: none;">Classification System </td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: none; padding: 10px;">IPC⁵</td> <td style="border: none; padding: 10px;">C 07 K, A 61 K</td> </tr> </table> <div style="text-align: center; margin-top: 10px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸</div>			Classification System	Classification Symbols	IPC ⁵	C 07 K, A 61 K											
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III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹ <table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 10%;">Category ¹⁰</th> <th style="width: 70%;">Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²</th> <th style="width: 20%;">Relevant to Claim No. ¹³</th> </tr> </thead> <tbody> <tr> <td style="text-align: center; vertical-align: top;">X</td> <td style="vertical-align: top;">EP, A, 0260610 (BASF AG) 23 March 1988 see page 3, lines 37-43</td> <td style="text-align: center; vertical-align: top;">1,2</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="text-align: center; vertical-align: top;">--</td> <td style="text-align: center; vertical-align: top;">3-7</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">Y</td> <td style="vertical-align: top;">US, A, 4676973 (M. BEST) 30 June 1987 see the whole document</td> <td style="text-align: center; vertical-align: top;">3-7</td> </tr> <tr> <td style="text-align: center; vertical-align: top;">P,X</td> <td style="vertical-align: top;">J. Clin. Invest., vol. 84, December 1989, The American Society for Clinical Investigation, Inc., (New York, US), J.S. Warren et al.: "Tumor necrosis factor participates in the pathogenesis of acute immune complex alveolitis in the rat",</td> <td style="text-align: center; vertical-align: top;">1-7</td> </tr> </tbody> </table>			Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³	X	EP, A, 0260610 (BASF AG) 23 March 1988 see page 3, lines 37-43	1,2	Y	--	3-7	Y	US, A, 4676973 (M. BEST) 30 June 1987 see the whole document	3-7	P,X	J. Clin. Invest., vol. 84, December 1989, The American Society for Clinical Investigation, Inc., (New York, US), J.S. Warren et al.: "Tumor necrosis factor participates in the pathogenesis of acute immune complex alveolitis in the rat",	1-7
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<div style="display: flex; justify-content: space-between;"> <div style="width: 48%;"> <p>¹⁰ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 48%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p> </div> </div>																	
IV. CERTIFICATION <table style="width: 100%; border: none;"> <tr> <td style="width: 50%; border: none; padding: 5px;"> Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">14th November 1990</div> </td> <td style="width: 50%; border: none; padding: 5px;"> Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">04.12.90</div> </td> </tr> <tr> <td style="border: none; padding: 5px;"> International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div> </td> <td style="border: none; padding: 5px;"> Signature of Authorized Officer <div style="text-align: center;">R.J. Eernisse </div> </td> </tr> </table>			Date of the Actual Completion of the International Search <div style="text-align: center; font-weight: bold;">14th November 1990</div>	Date of Mailing of this International Search Report <div style="text-align: center; font-weight: bold;">04.12.90</div>	International Searching Authority <div style="text-align: center; font-weight: bold;">EUROPEAN PATENT OFFICE</div>	Signature of Authorized Officer <div style="text-align: center;">R.J. Eernisse </div>											
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FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

pages 1873-1882,
see the abstract

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE ¹

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 8, 9, because they relate to subject matter not required to be searched by this Authority, namely:

See PCT-Rule 39.1 (IV); Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claim numbers..... because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers..... because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING ²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9001443

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/11/90. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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